CARDIOVASCULAR, PHARMACOLOGY and CHEMISTRY

#466R1
Activated: 6/1/65
Cf. #283
1/1/61 to 1/1/65

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

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NEW YORK, N. Y. 10017

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Application for Renewal of Research Grant
~~Application for Research Grant~~

Date: February 10, 1966

1. Name of Investigator: (1) Ebbe Curtis Hoff, Ph.D., M.D. (Principal Investigator)
(2) Karl C. Corley, Jr., Ph.D., (Co-Principal Investigator)

2. Title: (1) Professor and Chairman, Division of Psychiatric Research
(2) Assistant Professor, Division of Psychiatric Research

3. Institution & Address: Medical College of Virginia
1200 East Broad Street
Richmond, Virginia

4. Project or Subject: The Effects of Nicotine and its Intermediary Metabolites Upon the Central Nervous System, Autonomic Mechanisms, and Behavior.

5. Detailed Plan of Procedure (Use additional pages if more space is required.)

The following represents a Progress Report and additional Plan of Procedure.

The physiological section of the original application was divided into two parts: (1) Acute and (2) Chronic.

With the completion of the acute phases of the experiment during the previous report period, the major effort is being devoted to the chronic studies.

BEHAVIORAL STUDIES

(A) In accordance with the plan of procedure presented in the original proposal, studies on the effects of nicotine and metabolites upon food and water intake are in progress.

On food intake, six male Sprague-Dawley rats are being maintained on a 60-second fixed interval reinforcement schedule. When the baseline performance of the rat stabilize over a three day period with variability of less than ± 10%, the effect of subcutaneous doses of nicotine is tested. At doses as low as 20 micrograms per kilogram, a complete disruption of all behavior is observed 1 - 9 minutes after injection. When lever pressing returns, the level of responding appears to be similar to the control level. Difficulties, however, are encountered maintaining a reliable baseline behavior. At the earliest feasible time, the schedule will be changed to 60-second variable interval reinforcement. This schedule should provide a more reliable behavior, and show a clearer indication of the effects of nicotine.

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5. Detailed Plan of Procedure (Continued from Pg. 1)

On water intake, baseline data for daily consumption are now being obtained in six other rats. The protocol described in the original application is being followed, and the questions raised by the initial work will be explored.

(B) Additional experiments are also planned to study the effects of nicotine and its intermediary metabolites upon discrimination and learning behavior.

Recently an operant avoidance technique has been developed for the study of pattern discrimination in the rat. In discrete trials, random presentation of one of two pattern configurations requires discrimination between a right and left lever press to avoid shock.

The apparatus to establish this behavior consists of three Grason-Stadler multiple stimulus projectors for pattern presentation, two Lehigh Valley retractable levers for indication of response, and a grid floor for shock reinforcement. The projectors and levers are arranged so that there is a right and a left panel with a projector and a lever on both sides of a center panel with only a projector.

The pattern discrimination obtained in this experiment is maintained by a two phase schedule. The first phase and initial training involves a simple right versus left discrimination. Identical patterns are presented on the projectors of the center and one side panel. The correct response is always a lever press on the side panel showing a pattern. The second phase of the schedule is also presented on the previously unlit panel. This pattern is always different from the other two patterns, and the correct response is a lever press on the side panel showing a pattern corresponding with the center pattern and different from the pattern of the other side panel. In both phases, the presentations are randomized between the two pattern configurations by a pulse distribution.

In rats, highly trained on simple discrimination, the two phases of the schedule are combined to form an alternating mixed schedule. With the same patterns used in each phase of the schedule, every session begins with 15 minutes of the simple differentiation involving two patterns followed by 30 trials of the complex discrimination involving three patterns. Five cycles of this sequence or 150 trials involving three pattern discrimination comprise a session.

Regardless of the phase of the schedule involved, the trials consisted of the following sequence of events. Both levers are introduced into the box, and patterns are presented. In every trial, only a correct response terminates the trial, extinguishing the patterns and retracting the levers. If no correct lever press occurs within 50-seconds, shock is administered at 4-second intervals until the correct response occurs. During complex discrimination, involving three stimuli, the different third pattern is always extinguished after the first shock, thus reducing the configuration to simple discrimination involving two patterns.

During a trial incorrect responses always result in retraction of lever press. During complex discrimination and training of simple discrimination, no other consequences result from these responses, but in the simple dis-

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crimination phase of the mixed alternating schedule, these incorrect responses cause immediate commencement of the shock sequence that continues until the correct lever is pressed.

In fully trained rats, nicotine will be studied to determine its effect upon pattern discrimination. Because this technique uses avoidance procedures, the confounding effects related to the possible anorexic properties of nicotine will be avoided.

In another behavioral situation, the active learning and memory process will be studied. Rats will be trained on a two-lever discrimination. This task requires the association of one lever with food reinforcement and the other lever with no reinforcement. Within each session, the reinforcement contingencies of each lever are reversed after every fifth reinforcement. These reversals require the rat to constantly readapt its behavior pattern by learning and remembering the consequences of the previous lever press.

This adaptability is further emphasized by a delayed response contingency. After each lever press, the reinforcement does not occur until a sensing circuit is activated in the opposite end of the apparatus. At this point, the apparatus lights are extinguished for a specified time out period. If the correct lever was pressed, food reinforcement accompanies the commencement of the time out period, but an incorrect lever press results only in a time out. In both instances, lever presses during the time out are registered as incorrect response and restart the time out. This requires the rat to remember the consequences of its previous response and delay its next response until the apparatus lights are relit at the end of the time out.

In this situation, the duration of the time out has been found to be a critical variable. By analysis of the ratio between correct and incorrect lever presses, this factor can be studied. When the time out is very short (less than 5-second), random lever pressing occurs with no indication that the rat attempts to remember the correct lever from reinforcement to reinforcement. The same behavior is also observed with periods longer than 30 seconds. In between these extremes an optimal behavior is maintained with a time out of approximately 15-seconds.

Because this task provides a measure of the rat's ability to delay or withhold a correct response, a form of memory is being studied. If a compound influences memory, the length of the time that the rat can retain a correct response should be enhanced. Also if learning is affected, the number of correct responses should be reduced.

Previous experiments have shown that the effects of anorexic and stimulant agents can be separated from these other factors. With this ability of differentiation, it should be possible to determine if nicotine possesses any properties to enhance learning and/or memory.

In all of these behavioral situations, any consistent phenomenon found will be further studied with physiological and neurophysiological techniques. This approach makes it possible to explore and determine the neural correlates of these behaviors, and provide data on the mode and site of the action of nicotine.

EEG STUDIES

In cats with chronically implanted electrodes, the studies described in the original application are now in progress. The conditions and procedures previously outlined are being adhered to.

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6. Budget Plan:

a. Salaries	8,800.00
b. Expendable Supplies	2,504.00
c. Other Expenses	None
d. Permanent Equipment	2,000.00
e. Overhead (15% of a,b,c)	1,696.00
Total	15,000.00

7. Anticipated Duration of Work: It is anticipated that the duration of the studies outlined (under "Plan for Procedure") will continue for at least two more years following the end of present project year, i.e., May 31, 1966.

8. Facilities and Staff Available: The facilities available for research in our Division include: (1) a fully-equipped laboratory for acute studies; (2) a chronic laboratory with aseptic operating room and histology laboratory; (3) a laboratory with shielded cabinets where electrophysiological studies are conducted; (4) an electronic shop and instrument room; (5) a machine shop; (6) a darkroom; (7) a suite of rooms in which behavioral and neurophysiological studies are combined. The available staff includes the principal investigators, and in addition, three faculty members with special training in neurosurgery, neurology, neurophysiology, neuroanatomy and cardiology. There are also two full-time electronics specialists and three full-time research assistants trained in animal operative procedures. [redacted]

Standard neurophysiological and behavioral equipment is fully operational, and included Grass and Sanborn physiological recorders, an 8-channel and a 16-channel electroencephalograph, stimulators, stereotaxic instruments, telemetering equipment, apparatus for the construction of microelectrodes along with suitable recording equipment, solid state behavioral programming equipment, etc.

10. Additional Information (Including relation of work to other projects and other sources of support): This research has a significant contribution to make in the elucidation of the neural bases of behavior. A better understanding of the CNS and behavioral correlates of these mechanisms is essential for the interpretation and treatment of many psychosomatic disturbances. The studies outlined in this proposal may help elucidate the role of nicotine in motivation and learning.

All these experiments form an integral part of the continuing research program in our laboratories concerned with cerebral autonomic mechanisms and the regulation of food intake. Additional support is provided by research grants from NIH and ONR.

Signature [signature]
Director of Project

[signature]
Business Officer of the Institution
L. Daniel Crooks, Comptroller

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